A NEW RESPIRATION CALORIMETER

GEORGE J. PIERCE

It is generally known that heat is liberated, often in great quantity, whenever germination or fermentation takes place under such conditions that only a small proportion of the heat liberated is lost by radiation. For example, in the malting of barley, it is necessary to take precautions lest the temperature of the germinating grain rise too high; and California wine-makers have repeatedly told me that they are obliged to watch the temperature of the must very carefully lest the fermentation go too fast and "the yeast burn." Brewers and distillers are aware of the same fact. Botanists generally lecture about the liberation of heat in the spathes of aroids, but how many of us have seen an experiment demonstrating this phenomenon? I, for one, do not recall ever having seen one. I find that a considerable number of naturalists associate these various instances of heat liberation with rapid growth, and in some laboratory manuals of plant physiology the liberation of heat is treated in connection with growth phenomena. These same naturalists, and a good many others, think of respiration not only as involving an intake of oxygen (which is not always the case, as for example in anaerobic respiration), but also an outgo of carbon dioxid. This latter is by no means always the case, as the respiration of the sulphur, iron, and certain nitrogen bacteria shows.

The intake of oxygen and the outgo of carbon dioxid are the easiest features of the process of respiration to demonstrate in the organisms ordinarily studied in those biological laboratories in which any attention whatever is paid to live plants or animals. It is easy enough for us all to go on thinking of respiration as a sort of ventilating process, in which a poisonous waste product of the living organism is displaced or replaced by a useful gas. We do not necessarily realize how this latter gas is useful, except as it takes the place of another which is poisonous. Whether carbon dioxid is itself poisonous is, I suppose, open to dispute. At any rate, it is ordinarily of no use to the organism.

It can be used only as a food material, only by chlorophyll-containing cells, and by these only in the light.

The other features of the process of respiration are hard to demonstrate, hard even to study. The chemistry of respiration is scarcely less difficult than the chemistry of photosynthesis.[1] Whatever the reactions may be in the chain which connects the free oxygen of the air with the oxygen combined with carbon or iron or sulphur, etc., it is clear, on theoretical grounds, that the oxidation or oxidations which take place must liberate heat. The demonstration of this fact, with reasonably small quantities of living organisms, has hitherto been nearly impossible. For this reason, it has been almost useless for the teacher to insist that the liberation of heat, the setting-free of energy, and not the material products, is the essential end, the fundamental characteristic of respiration.[2]

The apparatus of BONNIER,[3] which has furnished the most satisfactory results so far in the study of the heat-yield, is too elaborate and too costly for most botanical laboratories. On the other hand, the simple appliances described and figured in the laboratory manuals are useless, for they do not illustrate the subject, but mislead the student. Using the ordinary apparatus and the usual quantities of live and dead peas, for example, one obtains, with good luck, a difference of 0°.5 C. between the live and dead peas in the course of twenty-four hours, or perhaps a whole degree, or, best of all, 1°.5! This is mortifying enough, but if the temperature of the laboratory fall much, so that all the peas are chilled, there will be practically no difference at all. The evidence of the experiments, therefore, is all against the teacher who would have it that respiration is the means of supplying the living thing with the energy it needs to do its work. Evidently the trouble is with the insulation of the vessels in which the respiring and the dead plants or parts are contained; for if radiation and absorption were reduced to a minimum, the live and respiring peas would certainly grow warmer, while the dead peas would remain at the same temperature or grow slightly cooler.

[1] See BARNES, C. R., The theory of respiration. BOT. GAZETTE **39**:81–98. 1905.

[2] See, for example, PEIRCE, G. J., Textbook of plant physiology, chap. 2.

[3] BONNIER, G., Recherches sur la chaleur végétale. Ann. Sci. Nat. Bot. VII. **18**: 1893.

Convinced of this, as well as thoroughly dissatisfied with the insulating appliances which I had or could make in the laboratory, I went to my friends in the chemical laboratory of this university, told them of my difficulty, and asked for suggestions. Professor YOUNG, professor of physical chemistry, suggested trying some Dewar flasks. I take this opportunity to acknowledge my indebtedness, and to express my gratitude to Mr. YOUNG, for his suggestion is one which will be appreciated by any physiologist who tries the apparatus for this purpose.

Dewar glassware is made in several shapes—cylinders, cups, and flasks. The flasks are made either tubulated or with closed round bottoms. The Dewar apparatus is made also silvered or unsilvered. The principle is simple—double-walled vessels with the air between the two walls exhausted. Thus there is a receptacle surrounded more or less completely by a vacuum. Across this vacuum radiation or absorption will take place at a rate inversely proportioned to the perfection of the vacuum. If the walls of the vessel are silvered on the surfaces bounding the vacuum, the efficiency of the insulation will be greatly increased. The apparatus was devised for liquid-air experiments and is named for the inventor, the famous chemist at the Royal Institution in London. This double-walled glassware has now come into commerce and may be bought, under the name of "thermal bottles," in drug stores and of the dealers in automobilists' and campers' supplies. It is used for keeping food or drink warm or cold, as may be desired, for many hours. Thus soup, milk, coffee, ice-water, etc., can be maintained at the desired temperature for astonishing lengths of time. The commercial bottles are protected against breakage by cases of metal or basketry, but since these do not improve the insulation materially, they are unnecessary in the laboratory, and the thinner-walled scientific apparatus is much cheaper, besides being obtainable in a greater variety of shapes.

In the experiments which I am about to describe, I used silvered Dewar flasks of about 250^{cc} capacity, which were supplied by EIMER and AMEND of New York for $2.40 each. If imported duty free, as they would have been had I not been impatient to use them, they would have cost decidedly less. There should be at least two such flasks, for it is desirable to use always a lot of dead or other check material

for comparison; but it is naturally better to set up the experiment in duplicate and thereby reduce the sources of error. In many instances I used three flasks of live material and one of dead. Since the efficiency of each flask as an insulator depends upon the completeness of the evacuation of the space between the walls and upon the silvering it is evident that the flasks themselves will not be exactly alike, and that an average result is likely to be better than any single one. If proper pains are taken, the efficiency of each flask can be determined in advance; but unless the experiments are conducted in a constant temperature, as they should be, there is little use in doing this.

Miss BERTHA A. WILTZ, a graduate student and assistant in physiology in this university, did most of the actual work of setting up and recording the results of these experiments; but as we have worked constantly together, the experiments are ours rather than the work of either one of us. As the work progressed, experience showed us how the experiments should be improved in method, but the reasons for these improvements will be more evident if I describe one.

Experiment 1.—An unweighed quantity of dry peas was soaked for 24 hours in tap-water. They were then rinsed in boiled distilled water two or three times and divided into two unequal lots, the smaller of which was then covered with a fairly concentrated aqueous solution of corrosive sublimate for at least half an hour in order to kill these peas. The other lot was divided into equal parts, which were poured into two Dewar flasks of about 250^{cc} capacity, the one silvered, the other unsilvered. The dead peas were poured into another silvered flask of the same size. The flasks were cotton-plugged and suspended on strings (not wire), in such a manner that they would not touch any object, metallic or other. This was done to avoid the changes in temperature which might otherwise result. A thermometer reading only to degrees was pushed through the cotton so that the bulb was as nearly as possible in the center of each mass of peas. The data will be found in the accompanying table (p. 197).

Various things are evident in this first rough experiment. It was continued for nearly nine days and, in spite of the fluctuating temperature of the room, the temperature in the silvered flask containing live peas rose until the last day very steadily. Comparing this with

the temperatures recorded for the unsilvered flask, containing live peas, and with the silvered flask into which dead (killed) peas had been placed, one sees at once the superiority of the silvered flask as an insulator, and also that there was liberated and retained in the silvered flask containing live peas a very substantial amount of energy in the form of heat, even within the first twenty-four hours. Even the unsilvered flask gave a result better than I had ever been able to obtain with the ordinary insulators available in the laboratory.

Date	Temp. live peas silvered	Temp. dead peas silvered	Temp. live peas unsilvered	Room temp.
Feb. 26, 4:30 P.M.	17	17	17	....
27, 8:15 A.M.	19	16	13	....
12:15 P.M.	20	16	16	....
4:00 P.M.	23	17.5	19	....
28, 9:20 A.M.	32	15.5	15.5	16
12:00 P.M.	33	16	....	....
5:00 P.M.	36	16	20	....
29, 9:20 A.M.	38	15	15	15.5
12:30 P.M.	39	15	17.5	18
3:30 P.M.	40	15.5	19	17
Mar. 1, 10:30 A.M.	42.5	15	17.5	16.5
2, 10:20 A.M.	45	14	15	16
1:30 P.M.	47.5	14	18	19
5:00 P.M.	49	14.5	20	19
3, 9:25 A.M.	53	14	15	16
1:00 P.M.	54	15	19	....
4:15 P.M.	54+	15.5	22	22
4, —— A.M.	54.5	15	14	14.5
4, 2:30 P.M.	55+	15	23	18
4:30 P.M.	56	15.5	23	19
5, 9:15 A.M.	54	14.5	14	13
12:30 P.M.	54	....	....	....
6, 10:00 A.M.	50	14	14	....

When the experiment was stopped on the ninth day, because the temperature had begun to fall, it was at once evident that fermentation had been very active for some time. It was inevitable that this should be so, for I had not taken the slightest pains to sterilize anything. But in fermentation and decay heat is liberated, for these are processes in which respiration, as well as nutrition, takes place. We have here, then, the heat liberated in the respiration of the peas and of the other organisms in the flask. The obvious thing to do in succeeding experiments is to sterilize the peas and everything else used in the experiment, and to make similar experiments to determine the heat liberated by various ferment organisms. This I have done, to a

very limited extent and only as a preliminary, with Fleischman's "compressed yeast," as will be shown later.

This first experiment, with all its roughness, seemed of sufficient importance to repeat several times in order to be quite sure that there was no mistake about it. The results were similar in every respect, and I do not need to record them here. The following experiment shows the effect of some of the improvements suggested by the preceding.

Experiment 2.—Into each of six Dewar flasks of approximately equal capacity, which had been sterilized by being washed with a saturated aqueous solution of corrosive sublimate and rinsed with boiled distilled water (sterile), 80^{gm} of air-dry peas were put. The peas and the flasks were then sterilized by shaking the peas very thoroughly in the flasks with a 1:500 aqueous solution of corrosive sublimate, and this was rinsed off with two wash-waters, both sterilized. Fresh sterilized distilled water was then poured into the flasks and the peas soaked in them for twenty-four hours at a temperature which ranged from 20 to 22° in the oven in which I had placed the flasks. I did not take the temperatures in the flasks during this time, as my apparatus was not then so arranged as to make that possible. However, I shall repeat the experiment under constant temperature and with readings from the beginning. The data will be found in the accompanying table (p. 199).

In this experiment fermentation and decay were reduced very greatly, though perhaps not as completely as possible. Therefore we have a very fair index of the amount of energy in the form of heat which 80^{gm} of peas (weighed air-dry) can liberate in something less than three days. We also see that, in all probability, the efficiency of the individual Dewar flasks varies considerably. The efficiency of each flask should be determined and recorded. I have not done this because it would be useless unless pains were also taken to conduct the experiments in a constant temperature, and this I was not able to do at that time. I shall repeat the experiment under uniform conditions as soon as possible to arrange it. The thermometers used in this experiment were good ones, reading to tenths of a degree, loaned to me by the department of physiology of this university, and I take pleasure in thanking my friend Professor

Date	Time	Flask 1	2	3	4	5 dead*	6	Room temp.	Max.	Min.
Apr. 29.....	6:00 P.M.	22°.7 C.	21°.6	22°.1	22°.4	22°.0	22°.3	19°.5		
Apr. 30.....	8:15 A.M.	23.7	22.3	23.8	23.8	18.2	23.0			
	12:15 P.M.	24.2	23.1	24.8	24.5	18.3	24.0	20.5		
	5:30 P.M.	27.0	25.7	27.3	27.4	19.6	26.5	21.0		
May 1.....	8:15 A.M.	31.1	31.7	32.4	32.4	17.4	31.3	14.5		
	12:30 P.M.	32.3	33.5	33.9	33.8	17.7	33.0	18.2		
	eve.	33.4	35.1	35.3	35.0	18.2	34.5	19.0	27°.5	14°.4
May 2.....	8:45 A.M.	35.7	38.4	39.0	37.5	17.6	37.5			
	12:30 P.M.	36.2	39.5	40.2	38.5	18.0	39.0			
	3:50 P.M.	36.8	40.5	41.4	38.8	18.4	40.0	18.3	26.2	16.1

Experiment stopped at 3:50 P.M., May 2.

	Flask 1	2	3	4	5	6	
No. peas decayed†.	3	2	4	2	0	3	Radicles 2.5–3^{cm} long.

Some of the sprouted peas planted in soil all grew normally.

* The peas marked *dead* were killed as in experiment 1.

† These decayed peas were without exception weevily, and because of the boreholes of the weevils could not be sterilized. Since the weevils close the outer ends of the holes, it is impossible to detect all the weevily peas when working with considerable quantities.

MACFARLAND for his help in this particular respect as well as in others. Owing to the position of the thermometers, making uniform sighting impossible, I do not record the attempt to read to fractions of tenths of degrees.

To see whether the heat liberated by a small amount of yeast in a small volume of a fermentable solution could be measured by the method described in the foregoing pages, I carried on some experiments, only one of which I need to report now. The yeast used was "Golden Gate compressed yeast," which is, I believe, only one of the many local names for what passes in the east under the name of Fleischman's compressed yeast. Four flasks were sterilized by being washed thoroughly with a saturated aqueous solution of corrosive sublimate. They were subsequently rinsed twice with sterilized water and plugged with cotton. Into each of these flasks 250^{cc} of 10 per cent. solution of cane sugar were poured as quickly as possible. The solution had previously been sterilized in four cotton-plugged flasks, from each of which it was poured into a Dewar flask. Since the yeast to be added is by no means a pure culture, I thought this sufficient care to exercise with the solution. To three of these Dewar

flasks approximately 4.5^{gm} of a yeast cake were added, to the fourth flask nothing. The yeast had been quickly rubbed up in a sterilized mortar, with a sterilized pestle, in a small quantity of the sterile sugar solution. Thus, on shaking the flask after adding the yeast, I hoped to mix the yeast thoroughly with the whole volume of fermentable liquid. The fourth flask contained, then, only sterilized sugar solution, which had been exposed to the air for only a moment in transferring it from one sterilized vessel to another. The data follow.

Date	Time	Flask 1	2	3	4	Room temp.
					(no yeast)	
April 8.....	6:00 P.M.	19°.1 C.	18°.6	18°.6	17°.8	
April 9.....	9:15 A.M.	22.1	20.5	21.1	17.5	
	5:30 P.M.	23.3	21.5	22.3	17.7	20°.0
April 10.....	8:00 A.M.	24.2	20.6	23.0	17.2	15.3
	12:30 P.M.	24.5	20.8	23.4	17.2	19.5
April 11.....	8:15 A.M.	25.0	20.6	24.0	17.3	
	12:30 P.M.	25.1	20.7	24.2	17.4	20.0

At this point, two days and eighteen hours after the experiment was started, it was ended and the flasks opened. The odor and flavor of the solutions were pleasant. I used the same thermometers as in the preceding experiment, but for the reasons previously given record here nothing less than tenths of degrees. Of course the thermometers were sterilized, by standing in saturated corrosive sublimate solution, and afterward washed in sterile water, before being introduced into the flasks. The efficiency of a good Dewar flask as an insulator is indicated by flask 4 in this experiment; for although the temperature of the room varied at least 5° C., as the record shows, the temperature within this flask varied only 0°.6 C., according to the readings taken at the same times. Whether flask 2 was a poor one, or whether the yeast was poor, or what the trouble was, I do not know. In the other two flasks the mercury rose to a degree which surprised me, considering the small amount of yeast sown in each of the three flasks and the small volume of fermentable liquid. This rise in temperature indicates the liberation of considerable energy in the form of heat.

In connection with these experiments on the respiration of healthy

plants I made one experiment, purely preliminary like the others, on wounded plants. RICHARDS reported in 1896 and 1897,[4] as a result of his experiments, that plants develop fever on wounding, as animals do. This increase in temperature is due to increased respiration in both sets of organisms. RICHARDS' methods are excellent, but unless an efficient insulator is used or the experiments are carried on in a constant temperature, they are not absolutely exact. I determined, therefore, to try Dewar flasks in a simple experiment of this sort. Two lots of onion sets (seedling onions) were carefully skinned, thus removing much if not all of the dead tissue by which these young bulbs are surrounded. Each lot was found to weigh 111^{gm} after skinning. One lot was put whole into a sterilized flask (no. 2), care being taken that the onions were not scratched or bruised in being put into the flask. The other lot was cut into irregular pieces, each onion into four to eight pieces, with an ordinary and fairly dull knife and put into another flask (no. 1). The temperature record follows.

Date	Time	Temp. no. 1 (chopped)	Temp. no. 2 (whole)	Room temp.
April 26.....	2:00 P.M.	17°5 C.	17°50	17°0
April 27.....	10:20 A.M.	23.25	18.50	19.0
	1:30 P.M.	25.00	19.50	....
	5:00 P.M.	27.00	20.25	20.0
April 28.....	12:15 P.M.	38.00	20.50	....

The experiment was stopped at noon, when it had run nearly two days, and the contents of each flask turned out upon the table. Both lots of onions appeared to be in perfectly normal condition. In some cases the edges of the cuts of the wounded onions were a little dry. The material looked as if the experiment could have been continued for twice this length of time without decay or other disturbance setting in. It would seem, therefore, that these flasks can be used for such experiments on wounded plants.

In the experiments here reported, temperatures are given as the evidence that energy (heat) is liberated in respiration. Although these temperatures are interesting, they do not give us any idea of the

[4] RICHARDS, H. M., Respiration of wounded plants. Annals of Botany 10:1896; 11:1897.

amount of heat liberated by a given organism or part. Because of the roughness of these preliminary experiments, and of my lack of the apparatus for carrying on the experiments under constant conditions, I have made as yet no effort to ascertain the number of calories liberated by a given weight of germinating peas. I hope to do this presently, not only for peas, but also for other things; but I do not wish this statement to be taken as suggesting that I wish to keep this method at present for my own use.

Dewar flasks seem to me to offer to the physiologist, both animal and plant, a convenient means of testing the yield of heat by respiration, testing in the case of an animal the calorific value of its food, testing in plants and plant parts the liberation of heat at various stages and under various conditions. Since the Dewar glassware is obtainable in various forms, and can be made in others if desired, it can be used for all the purposes of BONNIER'S experiments and for many others. For example, I see no reason why it would not be possible to ascertain the respiratory curve of any particular plant, from the beginning of the germination of its seeds until it had attained considerable size; to ascertain more exactly than we now know the relation of respiratory activity to the other activities or to the stages of development of the plant. These flasks, or cylinders, can be used also for demonstration experiments, on the lecture-table for example, proving at once to a class that respiration is really a process in which energy is released, and that it is the chief process by which the living organism obtains the energy which it constantly needs and uses.

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